

## SEQUENCE LISTINGS

<110> Hanmi Pharm. Co., Ltd.

<120> EXPRESSION VECTOR FOR SECRETING ANTIBODY FRAGMENT USING E. COLI SIGNAL  
SEQUENCE AND METHOD FOR MASS-PRODUCING ANTIBODY FRAGMENT

<130> PCA40739/HMY

<150> KR1020030072216

<151> 2003-10-16

<160> 36

<170> KopatentIn 1.71

<210> 1

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

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gggaagcttc gatcggacat ccagatgacc cagtctccat cctccctgtc tgcattctga 60

ggggacagag tcacc 75

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<211> 80

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<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

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tgcaatcagg ggtcccatct 80

<210> 4  
<211> 80  
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<220>  
<223> gene fragment of light chain variable region

<400> 4  
aggctgtagg ctgctgatgg tgagagtga atctgtccca gatccaçtgc cactgaaccg 60  
agatgggacc cctgattgca 80

<210> 5  
<211> 80  
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<223> gene fragment of light chain variable region

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<210> 6  
<211> 41  
<212> DNA  
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<223> gene fragment of light chain variable region

<400> 6  
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<210> 7  
<211> 75  
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<223> gene fragment of heavy chain variable region

<400> 7  
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aggtccctga gactc 75

<210> 8  
<211> 79  
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<223> gene fragment of heavy chain variable region

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<400> 8  
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gagtctcagg gacctgccg 79

<210> 9  
<211> 80  
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<223> gene fragment of heavy chain variable region

<400> 9  
tgcactgggt cggcaagct ccaggaagg gcctggaatg ggtctcagct atcacttgga 60  
atagtgtca catagactat 80

<210> 10  
<211> 80  
<212> DNA  
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<223> gene fragment of heavy chain variable region

<400> 10  
atacagggag ttcttggcgt tgtctctgga gatggtgaat cggccctcca cagagtccgc 60  
atagtctatg tgaccactat 80

<210> 11  
<211> 80  
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<223> gene fragment of heavy chain variable region

<400> 11  
acgccaagaa ctccctgtat ctgcaaatga acagtctgag agctgaggat acggccgtat 60  
attactgtgc gaaagtctcg 80

<210> 12  
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<212> DNA  
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<223> gene fragment of heavy chain variable region

<400> 12  
cactcgagac ggtgaccagg gtaccttggc cccaatagtc aagggaggac gcggtgctaa 60  
ggtacgagac tttcgcacag taat 84

<210> 13  
<211> 39  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> RT-PCR forward primer specific for heavy chain

<400> 13  
cccaagctta ggctccacc aagggcccat cggtcttcc 39

<210> 14  
<211> 48  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> RT-PCR reverse primer specific for heavy chain

<400> 14  
gggggatcct tatgggcacg gtgggcatgt gtgagttttg tcacaaga 48

<210> 15  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> RT-PCR forward primer specific for light chain

<400> 15  
cccaagcttt cgcgaactgt ggctgcacca tctgtcttca tc 42

<210> 16  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> RT-PCR reverse primer specific for light chain

<400> 16  
cccgatccc taacactctc ccctgttgaa gctctttgtg ac 42

<210> 17  
<211> 69  
<212> DNA  
<213> modified E. coli thermostable enterotoxin II signal sequence

<400> 17  
atgaaaaaga caatcgcat tcttcttgca tctatgttcg tttttctat tgctacaaat 60  
gcccaggcg 69

7

<210> 18  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing *Stu*I restriction enzyme site

<400> 18  
tctattgcta caaatgccca ggccttccca accattocct tatcc 45

<210> 19  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing *Stu*I restriction enzyme site

<400> 19  
agataacgat gtttacgggt ccggaagggt tggttaagga atagg 45

<210> 20  
<211> 51  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer specific for light chain

<400> 20  
gggggatacct cacgcggcgc atgtgtgagt ttgtcacaa gatttaggct c 51

<210> 21  
<211> 43  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing SD sequence and BamHI restriction enzyme site

<400> 21  
gggggatcca ggaggtgatt tatgaaaag acaatcgcat ttc 43

<210> 22  
<211> 44  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing BpuI restriction enzyme site

<400> 22  
ggggctgagc aggaggtgat ttatgaaaaa gacaatcgca ttcc 44

<210> 23  
<211> 52  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing BpuI restriction enzyme site

<400> 23  
ggggctcagc tcacgggagc catgtgtgag tttgtcaca agatttaggc tc 52

<210> 24  
<211> 63  
<212> DNA  
<213> E. coli OmpA signal sequence

<400> 24  
atgaaaaaga cagctatcgc gattgcagtg gcactggctg gtttcgctac cgttgcgcaa 60



gct

63

&lt;210&gt; 25

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; forward primer specific for heavy chain

&lt;400&gt; 25

gagggttcagc tagtcgagtc aggaggcggt

30

&lt;210&gt; 26

&lt;211&gt; 51

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; forward primer containing HindIII and StuI restriction enzyme sites

&lt;400&gt; 26

gggagatctt cacgcggcgc atgtgtgagt ttgtgcacaa gatctaggct c

51

&lt;210&gt; 27

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; reverse primer containing stop codon and BamHI restriction enzyme site

&lt;400&gt; 27

gacattcaaa tgaccagag cccatccagc

30

&lt;210&gt; 28

10

<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> forward primer containing HindIII and NruI restriction enzyme sites

<400> 28  
cccagatctc taacactctc cctgttgaa gctctttgtg ac 42

<210> 29  
<211> 41  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 29  
ggggtcgaca ggaggtgatt tatgaaaaag acagctatcg c 41

<210> 30  
<211> 51  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> reverse primer containing SalI restriction enzyme site

<400> 30  
ggggtcgact cagcggcgc atgtgtgagt ttgtcaca gatttaggct c 51

<210> 31  
<211> 42  
<212> DNA  
<213> Artificial Sequence

11

&lt;220&gt;

<223> forward primer specific for modified E. coli enterotoxin II signal peptide and containing NdeI restriction enzyme site

&lt;400&gt; 31

gggcataatga aaaagacaat cgcatttctt cttgcatcta tg

42

&lt;210&gt; 32

&lt;211&gt; 705

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; TNF-alpha heavy chain

&lt;400&gt; 32

gaggttcagc tagtcgagtc aggaggcggg ttggtacagc ccggcaggtc cctgagactc 60

tcctgtgcgg cctctggatt cacctttgat gattatgcc a tgcactgggt ccggcaagct 120

ccaggaagg gcctggaatg ggtctcagct atcacttgg a atagtggta catagactat 180

gcggactctg tggagggccg attcaccatc tccagagaca acgccaagaa ctccctgtat 240

ctgcaaatga acagtctgag agctgaggat acggccgtat attactgtgc gaaagtctcg 300

taccttagca ccgcgtcctc ccttgactat tggggccaag gtaccctggt caccgtctcg 360

agtgcctcca ccaagggccc atcgggtctc cccctggcac cctcctccaa gagcacctct 420

gggggcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480

tcgtggaact caggcgcctt gaccagcggc gtgcacacct tccggctgt cctacagtcc 540

tcaggactct actccctcag cagcgtgggt accgtgccct ccagcagctt gggcaccag 600

acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag 660

cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc catag 705

12

<210> 33  
 <211> 645  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> TNF-alpha light chain

<400> 33  
 gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtagggga cagagtcacc 60  
 atcacttgtc gggcaagtca gggcatcaga aattacttag cctggatatca gcaaaaacca 120  
 gggaaagccc ctaagctcct gatctatgct gcatccactt tgcaatcagg ggtcccatct 180  
 cggttcagtg gcagtggatc tgggacagat ttactctca ccatcagcag cctacagcct 240  
 gaagatggtg caacttatta ctgtcaaagg tataaccgtg caccgtatac ttttggccag 300  
 gggaccaagg tggaaatcaa acgaactgtg gctgcaccat ctgtcttcat ctcccgcca 360  
 tctgatgagc agttgaaatc tggaaactgcc tctgttgtgt gcctgctgaa taacttctat 420  
 cccagagagg ccaaagtaca gtggaagggtg gataacgccc tccaatcggg taactcccag 480  
 gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540  
 ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600  
 ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag 645

<210> 34  
 <211> 7  
 <212> PRT  
 <213> TNF-alpha light chain

<400> 34  
 Asp Ile Gln Met Thr Gln Ser  
 1 5

13

<210> 35  
<211> 8  
<212> PRT  
<213> TNF-alpha heavy chain

<400> 35  
Glu Val Gln Leu Glu Val Asp Ser  
1 5

<210> 36  
<211> 12  
<212> PRT  
<213> N-terminal sequence of recombinant TNF-alpha

<400> 36  
Asp Glu Ile Val Gln Met Leu Thr Val Gln Asp Ser  
1 5 10